Supplementary Methods

Transplantation of LSK cells

For serial transplantation of LSK cells, $1x10^5$ LSK cells from C57BL/6 mice transduced twice with *pMYs-Setbp1-IRES-GFP* or *pMYs-IRES-GFP* virus were first transplanted into each lethally irradiated primary B6-*Ly5.2* recipient along with 7.5×10^5 supporting bone marrow cells. At 4 months after primary transplantation, 5×10^2 GFP^+ LSK cells purified from the primary recipients by FACS were transplanted into each lethally irradiated secondary B6-*Ly5.2* recipient along with supporting bone marrow.

In vitro HDAC inhibitor treatment

5×10^5 *Setbp1*-induced leukemic cells (BL3 and BL12) plated in media (IMDM, 20% horse serum and 1x penicillin/streptomycin) with SCF (50ng/ml) and IL3 (10ng/ml) were treated with 1μM of Entinostat (LC Laboratories Woburn, MA and Selleck Chemicals, Houston, TX), Vorinostat (LC Laboratories) or equal volume of control DMSO for 48hrs. Treated cells were subsequently subjected to cytospin, RNA extraction and Western blotting analysis. For colony formation assay, $2x10^4$ BL3 and BL12 cells were plated in IMDM methylcellulose medium supplemented with 20% horse serum, mouse SCF (50ng/ml), IL-3 (10ng/ml) and 1μM of Entinostat, Vorinostat, or DMSO. Colony numbers were counted after 7 days.

In vivo Entinostat treatment

Spleen cells from Setbp1-induced leukemic mice (BL12 and BL19) were transplanted into lethally irradiated secondary recipients ($1x10^6$ cells/animal) for inducing leukemia

development. Beginning 7days after transplantation, recipient mice were injected intraperitoneally with either 30 mg/kg of Entinostat (dissolved in 20 µl of DMSO and 180 µl of 50% polyethylene glycol) or vehicle once every 3 days for 21 days.

Lentiviral production, infection, and analysis

pLKO.1 lentiviral constructs containing shRNAs were purchased from Sigma Aldrich (NC-sh, SHC002; GFP-sh, SHC005; Hdac1-sh1, TRCN0000039402; Hdac1-sh2, TRCN0000039403; St. Louis, MO) and infectious lentivirus were generated as described previously. [1] Colony formation assays were performed at 48 hours after infection using 2 $x10^4$ puromycin-resistant cells on IMDM methylcellulose medium supplemented with 20% horse serum, mouse SCF (50ng/ml) and IL-3 (10ng/ml), and puromycin (2 µg/ml). Colony numbers were counted after 7days.

Western blotting analysis

For Western blotting analysis, cells were washed twice with cold PBS and then whole cell lysates were prepared by direct lysis of cell pellets in heated 2 x SDS sample buffer. Samples were resolved on 4-12% tris-glycine gels (Life Technologies, Carlsbad, CA) before transferring onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Primary antibodies used include anti-Setbp1 (16841-1AP, Proteintech, Chicago, IL)[1], Runx1 (19555-1-AP, Proteintech) and β-actin (MAB1501R, Millipore). Secondary antibodies used include goat anti-rabbit (SC-2004, Santa Cruz Biotechnology, Dallas, TX) and anti-mouse IgG-HRP (a-9044, Sigma Aldrich). Protein bands were visualized by incubation

with SuperSignal West chemiluminescent substrate (Pierce, Thermo Fisher Scientific, Rockford, IL) and quantified using Quantity One data analysis software (Bio-Rad).

Real-time RT-PCR

For real-time RT-PCR, total RNA was extracted from cells using RNAeasy Plus mini kit (QIAGEN, Germantown, MD). Oligo-dT-primed cDNA samples were prepared using Superscript III (Invitrogen), and real-time PCR analysis was performed in triplicates using SYBR green detection reagents (Invitrogen) on a 7500 real time PCR system (Applied Biosystems). Relative changes in expression were calculated according to the $^{\Delta\Delta}$Ct method. The cycling conditions were 50°C for 2 minutes followed by 95°C for 2 minutes, and then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The following gene-specific primer sequences were used:

Setbp1	5’ CTG CTC ACT GTG GAG ACG ATT C 3’ 5’ TTC TTA TCC AGC ACA CCA AGC TT 3’
Hoxa9	5’ TGT CTC CTC TCC CCC AAA CC 3’ 5’ GAG ATG AGG CCT GGG ATTTAG A 3’
Hoxa10	5’ CCA GCC CTG GGT AAA CTT AGC 3’ 5’ CATTGA CCT CAG GCC AGA CA 3’
Runx1	5’ GCA GGC AAC GAT GAA AAC TAC T 3’ 5’ GCA ACT TGT GGC GGA TTT GTA 3’
β–Actin	5’ CCT CCC TGG AGA AGA GCT A 3’; 5’ TCC ATA CCC AAG AAG GAA G 3’
Rpl4	5’ ATG ATG AAC ACC GAC CTT AGC A 3’

	5’ CGG AGG GCT CTT TGG ATT TC 3’
Cd11b	5’ GAA GCT GCC CCC CAA GAC 3’ 5’ GGT CAA TGC ATG GAG AAA AGG 3’
Csf1r	5’ TGG ACT TCG CCC TCA GCT T 3’ 5’ CCC CAG ACC CCT CAT GTT C 3’
Lyz2	5’ TGT GAG CTG CAG GGC TTT G 3’ 5’ CCC ACC ACA GAG GCT GTT CT 3’
Mta2	5’ TGT ACC GGG TGG GAG ATT AC 3’ 5’ CCT TCG CCG GAA AAG ACA G 3’
Mbd3	5’ CCC CAG CGG GAA GAA GTT C 3’ 5’ CGG AAG TCG AAG GTG CTG AG 3’
RUNX1	5’ TTT GTC GGT CGA AGT GGA AGA 3’ 5’ GAT TTT GAT GGC TCT GTG GTA GGT 3’
SETBP1	5’ GCT CAC AGT CGA GAC GAT TCA T 3’ 5’ AAT TGC GTC GTC GCT TTC TT 3’
β–ACTIN	5’ CCT GGC ACC CAG CAC AAT 3’ 5’ GCC GAT CCA CAC GGA GTA CT 3’

Chromatin immunoprecipitation (**ChIP)**

Mouse myeloid progenitors immortalized by FLAG-tagged *Setbp1* were generated as described. [1] ChIP analyses were performed using ChIP-IT Express kit (Active Motif). Immunoprecipitations were performed using FLAG M2 (Sigma Aldrich), mouse monoclonal anti-HDAC1 antibody (10E2, #5356, Cell Signaling Technologies) [2], rabbit

polyclonal anti-acetylated histone H3 (#39139, Active Motif) [3], rabbit polyclonal anti-Mta2 (sc-28731, Santa Cruz), rabbit polyclonal anti-Mbd3 (A302-528A, Bethyl), mouse IgG (G3A1, #5415, Cell Signaling Technologies), rabbit polyclonal anti-Chd4 (14173-1, Proteintech), and rabbit IgG (#P120-101, Bethyl Laboratories). Chromatin DNA was purified using MinElute PCR Purification Kit (QIAGEN) and quantified by real-time PCR. The following *Runx1* promoter-specific primers were used:

Runx1-P1	5' ACA GGA TCT GAA AGC CAC CAA 3' 5' CCT GCC TCA GTC TTT TCT TGC T 3'
Runx1-P2	5' CCG TCG GTC TCC TCT ATG CA 3' 5' GCC CGA CCC GAG GAA TT 3'
Neg	5' GTT AGC CTT TCA TGT CTG TGG 3' 5' GAT CAA TGC TCT GAC ATC AGA G 3'

Immunoprecipitation

Nuclear extracts were harvested from 293T cells at 36 hours after transient transfection with an expression construct expressing 3xFLAG-tagged Setbp1, and immunoprecipitation was carried out with FLAG M2 or a Mta2-specific antibody (Abcam) using the Nuclear Complex Co-IP kit (Active Motif). Immunoprecipitates were analyzed by Western blotting analyses using antibodies as described for ChIP.

Southern blotting analysis

7 ug of DNA from leukemic spleens were digested with *EcoRI*, resolved on 0.75% agarose gel, and transferred to nylon membrane using standard procedures. ^{32}P-

labeled *GFP*-specific probe was synthesized by random primer labeling using the Prime-IT II kit (Stratagene, LoJolla, CA) and hybridization was carried out in MiracleHyb buffer (Stratagene) following the manufacturer's instructions.

Statistical analysis

Sample sizes and animal numbers were determined by previous experiences. No samples were excluded from analyses. All data were analyzed by two-tailed Student's *t*-test except that survival curves were compared by Log-rank test. The researchers were not blinded during sample collection and analysis.

Supplementary Data

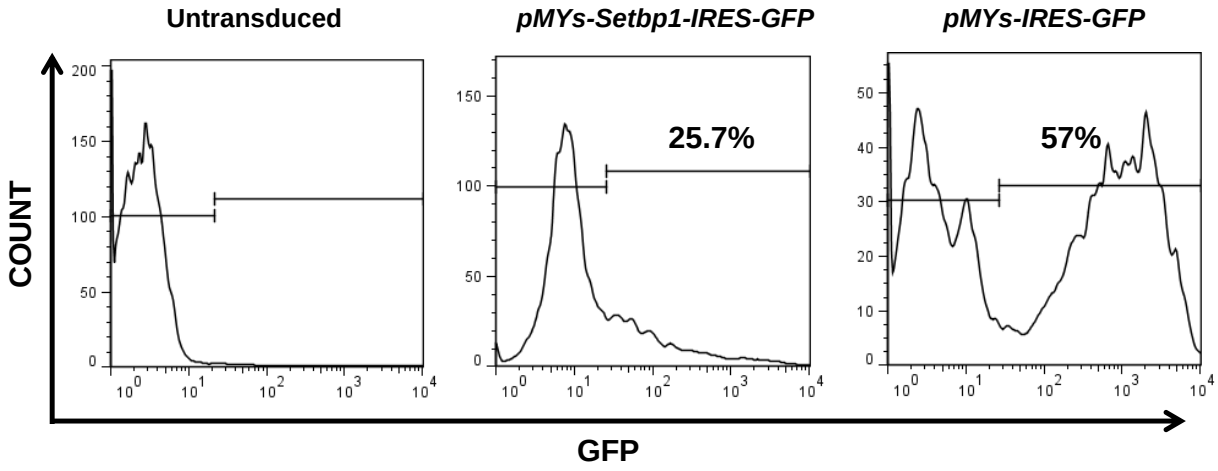

Figure S1. Transduction efficiencies for 5-FU treated mouse bone marrow progenitors. Representative transduction efficiencies in indicated transduction groups determined by GFP fluorescence are shown. 20-50% and 55-72% infection efficiencies were observed for *pMYs-Setbp1-IRES-GFP* and *pMYs-IRES-GFP* virus respectively. Samples were analyzed at 48 hours after infection. Numbers represent the percentages of GFP positive cells.

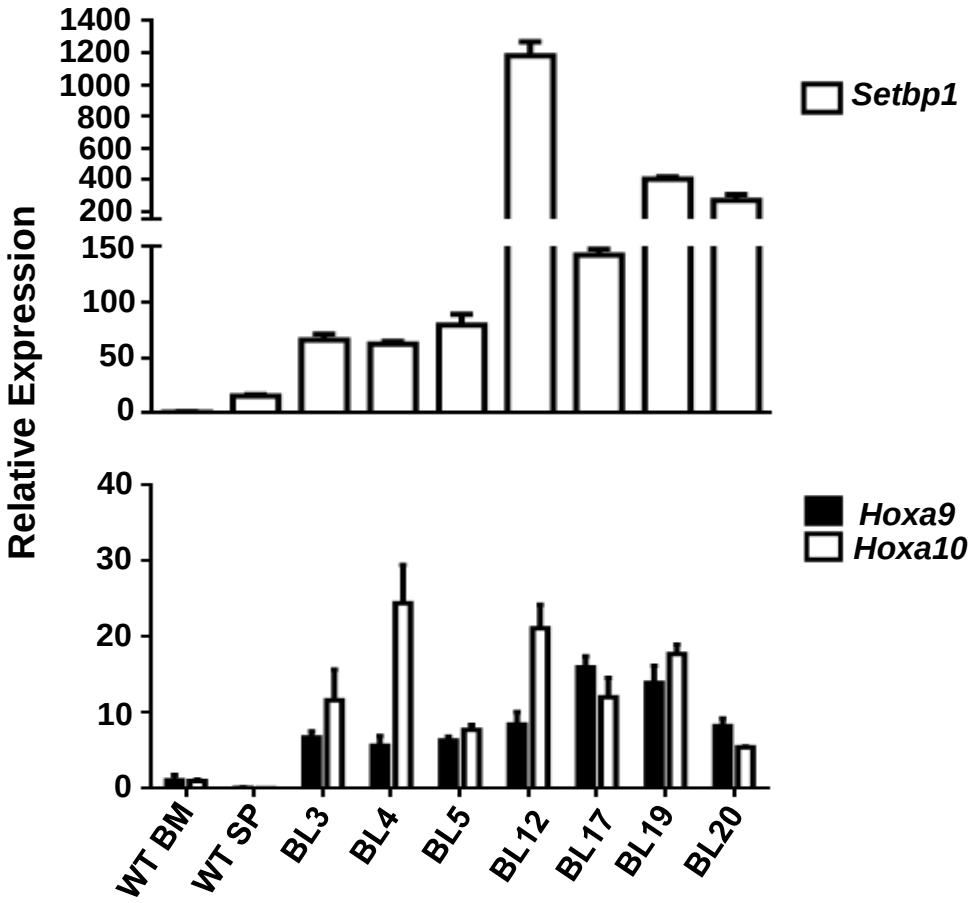

Figure S2. Increased expression of *Setbp1*, *Hoxa9* and *Hoxa10* in *Setbp1*-induced myeloid leukemias. Real-time RT-PCR analysis of total RNA extracted from spleens of Setbp1-induced leukemic mice (BL3, BL4, BL5, BL12, BL17, BL19 and BL20) and control normal bone marrow (BM) and spleen (SP) using gene-specific primers (n=3). Relative expression levels were calculated by normalizing to *Rpl4* mRNA levels in the same sample and also wild-type bone marrow. The mean and SD of each relative expression level is shown.

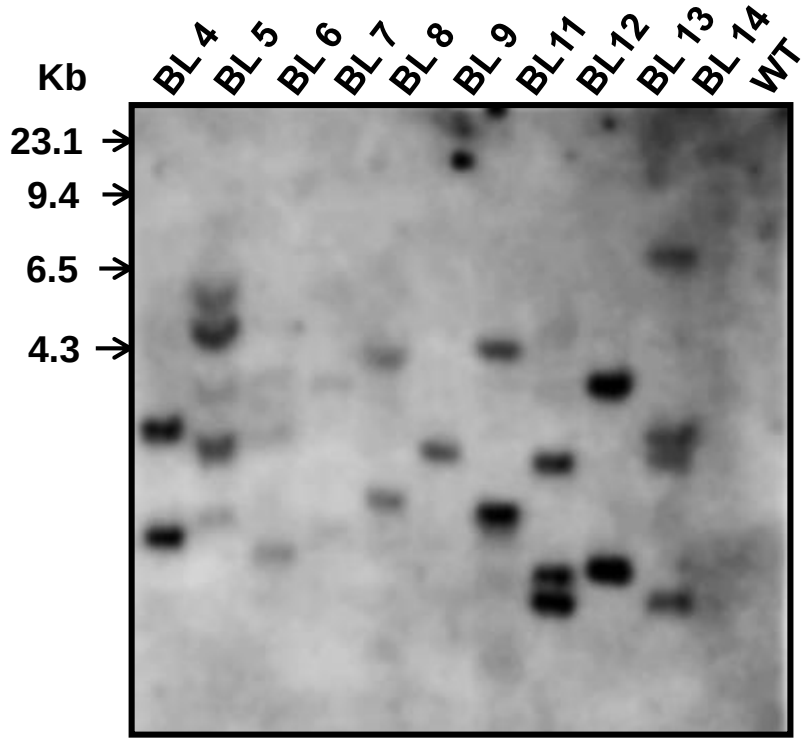

Figure S3. *Setbp1*-induced leukemias are mostly clonal. Southern blotting analysis of viral integrations present in 10 *Setbp1*-induced myeloid leukemias (BL4-9, BL11-14) using a *GFP*-specific probe. Seven ug of genomic DNA from each leukemic spleen was digested with*EcoR*I, resulting the generation of a single *GFP*-containing DNA fragment from each provirus. Each band represents an independent integration. Same amount of genomic DNA from wild-type spleen (WT) was included as negative control.

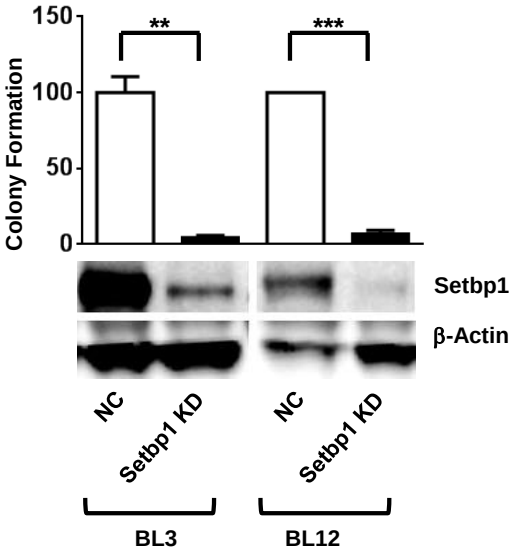

Figure S4. *Setbp1*-induced leukemia cells are dependent on *Setbp1* expression for maintenance. Upper panel, mean and SD of colony-forming potential of *Setbp1*-induced leukemia cell lines BL3 and BL12 in the presence of SCF and IL-3 at 48 hours after infection with pLKO.1 lentiviral shRNAs targeting GFP and Setbp1 (Setbp1KD) or control lentiviral shRNA (NC). [1] Lower panel, representative Western blotting analyses of Setbp1 and β-Actin protein levels in the infected cells of the top panel at 72 hours after infection. **, $P < 0.01$; ***, $P < 0.001$ (two-tailed Student's *t* test).

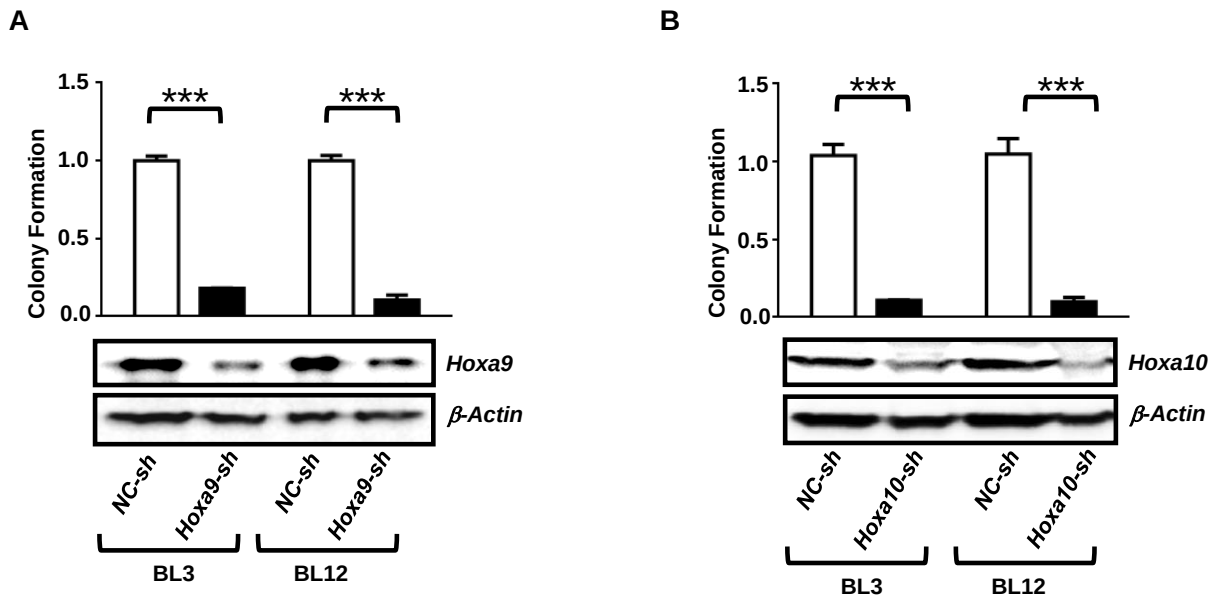

Figure S5. *Setbp1*-induced leukemia cells are dependent on *Hoxa9 and Hoxa10* expression for maintenance. Upper panel, mean and SD of colony-forming potential of *Setbp1*-induced leukemia cell lines BL3 and BL12 in the presence of SCF and IL-3 at 48 hours after infection with *Hoxa9*-specific lentiviral shRNA (A, *Hoxa9-sh*) or *Hoxa10*-specific lentiviral shRNA (B, *Hoxa10-sh*) in comparison to control lentiviral shRNA (NC-sh). Both *Hoxa9* and *Hoxa10* shRNAs have been validated previously. [1] Lower panels, representative Western blotting analyses of indicated proteins in the infected cells of the top panel at 72 hours after infection. ***, $P < 0.001$ (two-tailed Student's *t* test).

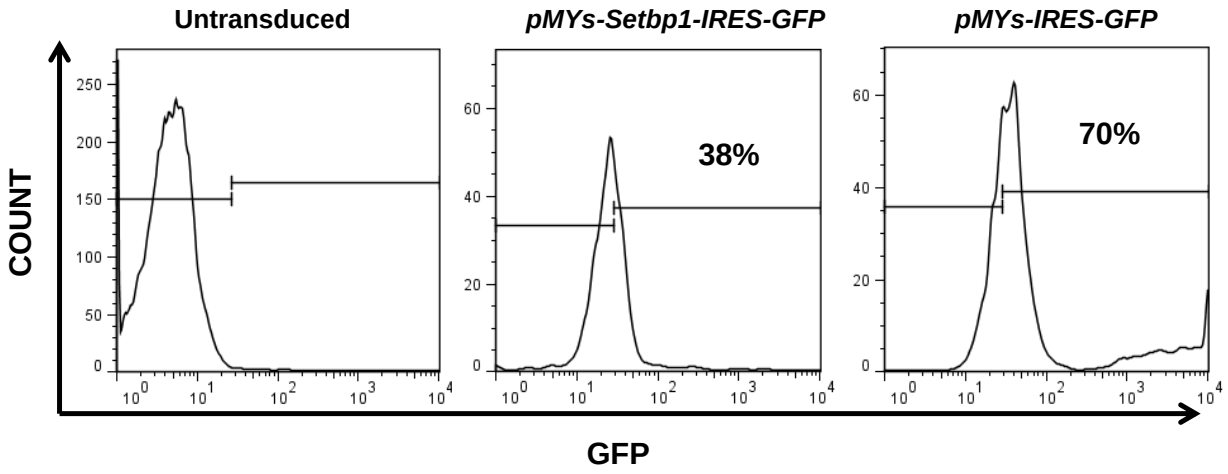

Figure S6. Transduction efficiencies for purified mouse LSK cells. Representative transduction efficiencies in indicated transduction groups were determined by GFP fluorescence. 37-75% and 70-85% infection efficiencies were observed for *pMYs-Setbp1-IRES-GFP* and *pMYs-IRES-GFP* virus respectively. Samples were analyzed at 48 hours after infection. Numbers represent the percentages of GFP positive cells.

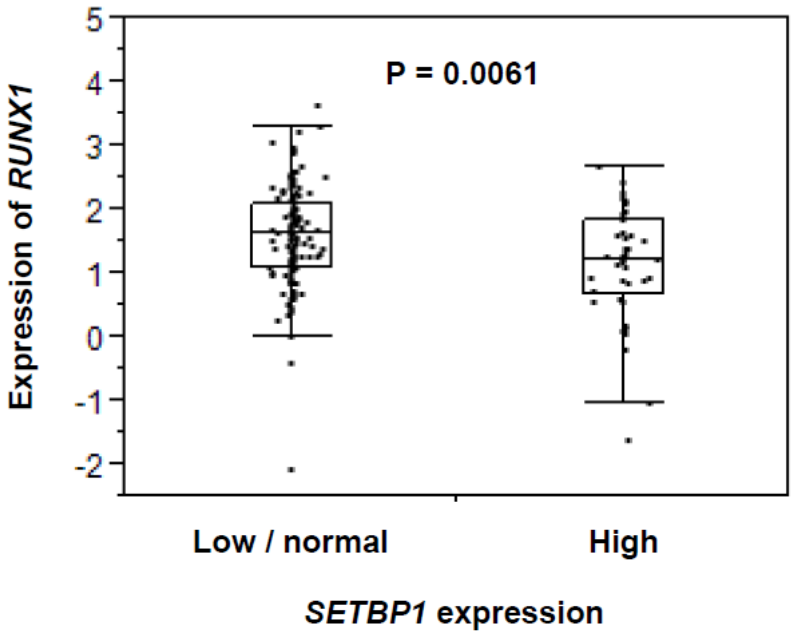

Figure S7. Correlation between *SETBP1* and *RUNX1* expression in human AMLs. Expression array values were extracted from Oncomine dataset[4]. p-values were calculated by comparisons between indicated 2 groups with AML (without *RUNX1* mutations) using Mann-Whitney *U* test. Cut off value of high (n=42) and low / normal (n=140) expression of *SETBP1* was mean+0.5 standard deviation.

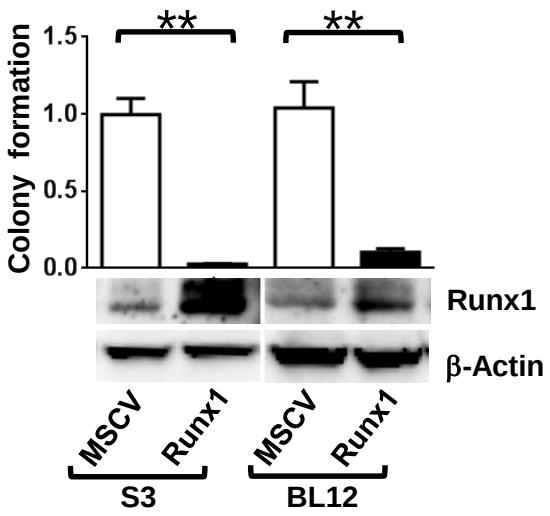

Figure S8. Ectopic *Runx1* expression inhibited colony formation by *Setbp1*-induced immortalized and leukemia cells. Upper Panel , mean and s.d. of colony formation potential of S3 and BL12 cells after infection with *MSCV-Runx1* (*Runx1*) or control empty *MSCV* (*MSCV*) virus (n=3 for each infection). Lower panels, representative Western blotting analysis of Runx1 and β-actin protein levels at 72 hrs after infection. **, $P < 0.01$ (two-tailed Student's *t* test).

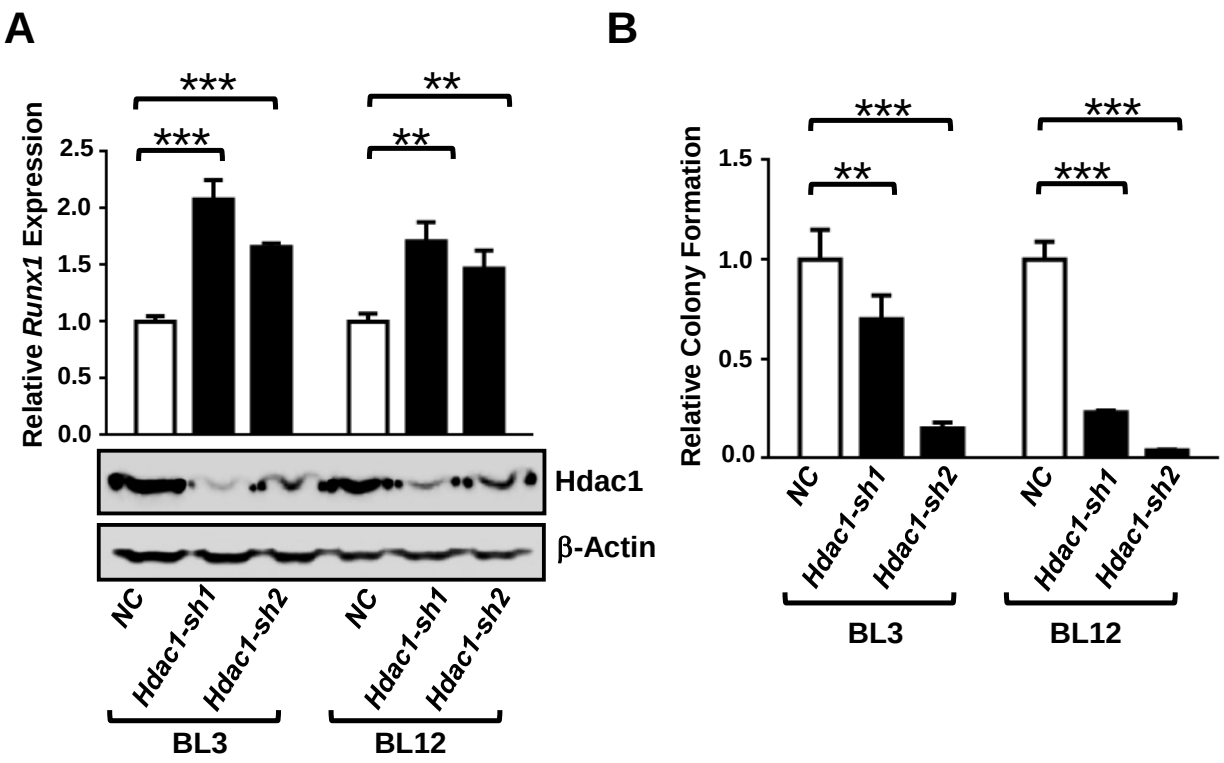

Figure S9. *Hdac1* knockdown increased *Runx1* mRNA levels and inhibited colony formation of *Setbp1*-induced leukemia cells. **(A)** Top panel, real-time PCR analysis of *Runx1* mRNA levels in *Setbp1*-induced leukemia cells BL3 and BL12 at 72hrs after infection with lentiviral shRNAs targeting *Hdac1* (*Hdac1-sh1* and *-sh2*) or control (NC) shRNA (n=3 for each infection). Relative expression levels were calculated by normalizing to *Rpl4* mRNA levels in the same sample and also control (NC) sample. The mean and s.d. of each relative expression level is shown. Lower panels, representative Western blotting analyses of Hdac1 and β-Actin protein levels in the infected cells of top panel at 72 hours after infection. **(B)** Mean and s.d. of colony-forming potential of *Setbp1*-induced leukemia cells BL3 and BL12 in the presence of SCF and IL-3 at 48 hours after infection with lentiviral shRNAs as shown in (C). **, $P < 0.01$; ***, $P < 0.001$ (two-tailed Student's *t* test).

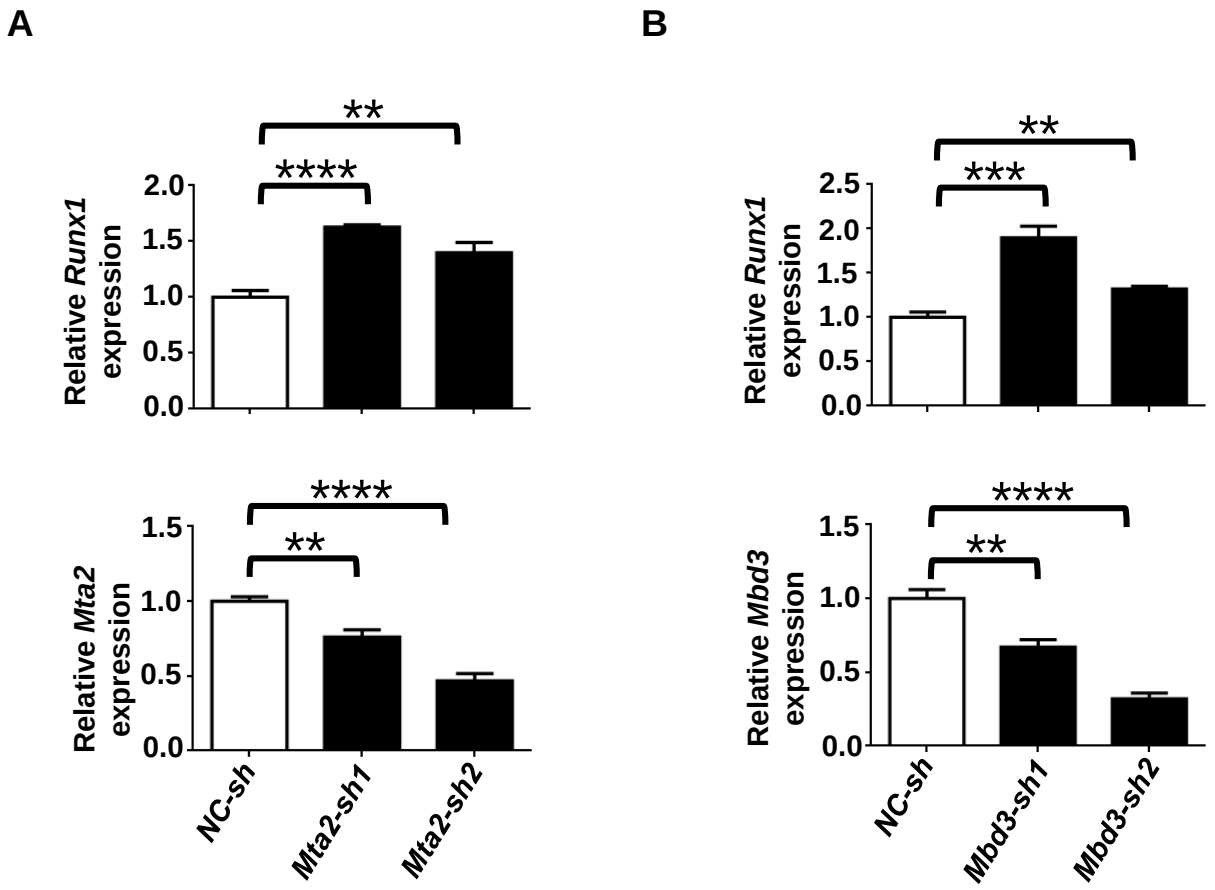

Figure S10. *Mta2 and Mbd3* knockdown increased *Runx1* transcription in *Setbp1*-induced BL3 leukemia cells. **(A)**. Real-time PCR analysis of *Runx1* (top panel) and *Mta2* (lower panel) mRNA levels in *Setbp1*-induced leukemia cells BL3 at 72hrs after infection with pLKO.1 lentiviral shRNAs targeting *Mta2* (*Mta2-sh1* and *-sh2*) or control (NC) shRNA (n=3 for each infection). **(B)**. Real-time PCR analysis of *Runx1* (top panel) and *Mbd3* (lower panel) mRNA levels in *Setbp1*-induced leukemia cells BL3 at 72hrs after infection with pLKO.1 lentiviral shRNAs targeting *Mbd3* (*Mbd3-sh1* and *-sh2*) or control (NC) shRNA (n=3 for each infection). pLKO.1 lentiviral constructs containing shRNAs were purchased from Sigma Aldrich (NC-sh, SHC002; *Mta2-sh1*, TRCN0000039355;

Mta2-sh2, TRCN0000039358; *Mbd3-sh1*, TRCN0000039069; *Mbd3-sh2*, TRCN0000039072). Relative expression levels were calculated by normalizing to *Rpl4* mRNA levels in the same sample and also control (NC-sh) sample. The mean and SD of each relative expression level is shown. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ (two-tailed Student's *t* test).

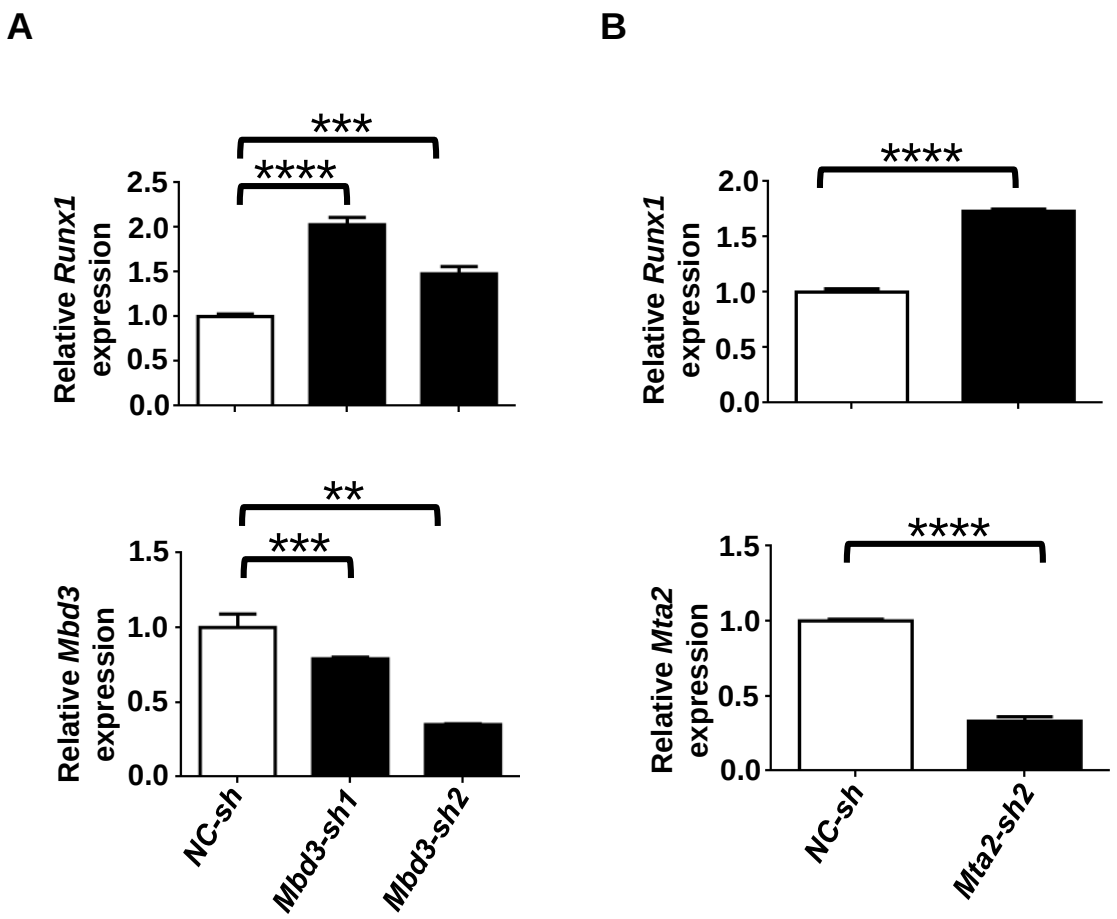

Figure S11. *Mbd3 and Mta2* knockdown increased *Runx1* transcription in *Setbp1*-induced BL12 leukemia cells. **(A)**. Real-time PCR analysis of *Runx1* (top panel) and *Mbd3* (lower panel) mRNA levels in *Setbp1*-induced leukemia cells BL12 at 72hrs after infection with pLKO.1 lentiviral shRNAs targeting *Mbd3* (*Mbd3-sh1* and *-sh2*) or control (NC) shRNA (n=3 for each infection). **(B)**. Real-time PCR analysis of *Runx1* (top panel) and *Mta2* (lower panel) mRNA levels in *Setbp1*-induced leukemia cells BL12 at 72hrs after infection with pLKO.1 lentiviral shRNAs targeting *Mta2* (*Mta2-sh2*) or control (NC) shRNA (n=3 for each infection). pLKO.1 lentiviral constructs containing shRNAs were purchased from Sigma Aldrich (NC-sh, SHC002; *Mbd3-sh1*, TRCN0000039069; *Mbd3-sh2*, TRCN0000039072; *Mta2-sh2*, TRCN0000039358). Relative expression levels

were calculated by normalizing to *Rpl4* mRNA levels in the same sample and also control (NC-sh) sample. The mean and SD of each relative expression level is shown. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ (two-tailed Student's *t* test).

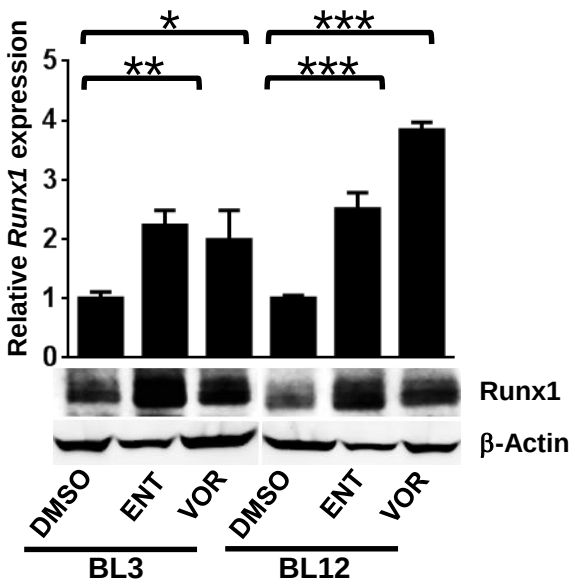

Figure S12. HDAC inhibition increased *Runx1* expression in *Setbp1*-induced leukemia cells. Upper panel, real-time RT-PCR analysis of *Runx1* mRNA levels using total RNA from indicated *Setbp1*-induced leukemic cell lines 48 hours after treatment with 1μM of Entinostat (ENT) or Vorinostat (VOR) in comparison to DMSO treated control. Relative expression levels were calculated by normalizing to β-*Actin* mRNA levels (n=3 for each treatment). Lower panel, representative Western blotting analysis of Runx1 and β-Actin protein levels in the same cells. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (two-tailed Student's *t* test).

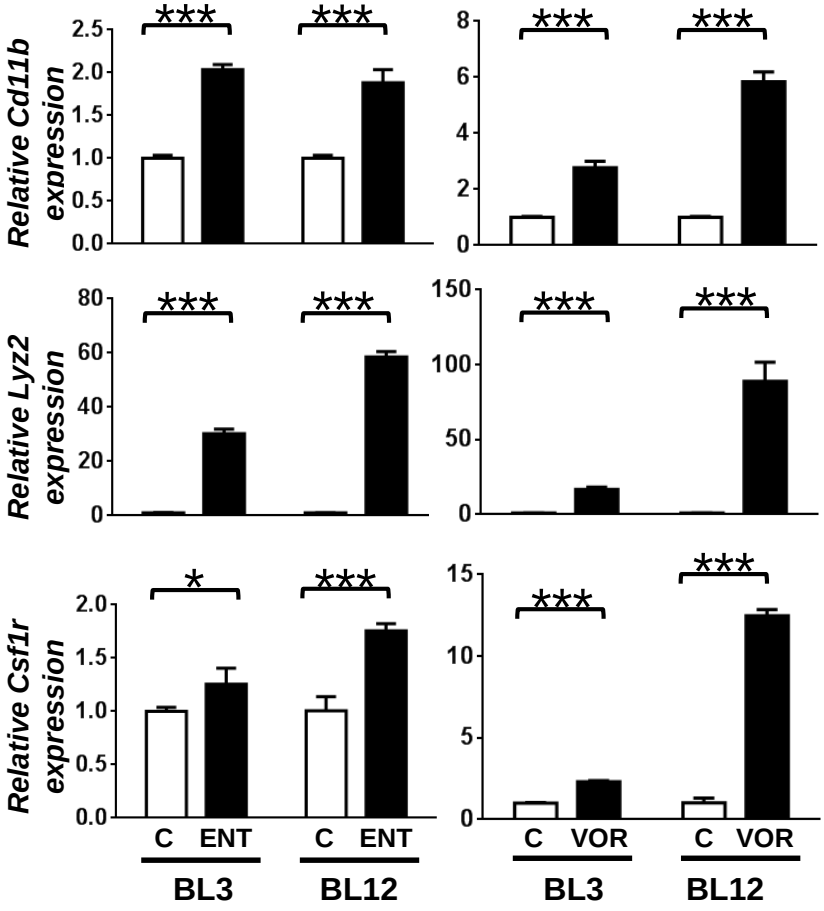

Figure S13. HDAC inhibition promoted differentiation of *Setbp1*-induced myeloid leukemia cells. Real-time RT-PCR analysis of total RNA from BL3 and BL12 cells at 48 hours after treatment with 1μM entinostat (ENT), vorinostat (VOR) or DMSO (C) using primers specific for myeloid differentiation marker genes *Cd11b*, *Lyz2* or *Csf1r*. Data are shown as means ± s.d. (n=3 for each treatment). *, $P < 0.05$; ***, $P < 0.001$ (two-tailed Student's *t* test).

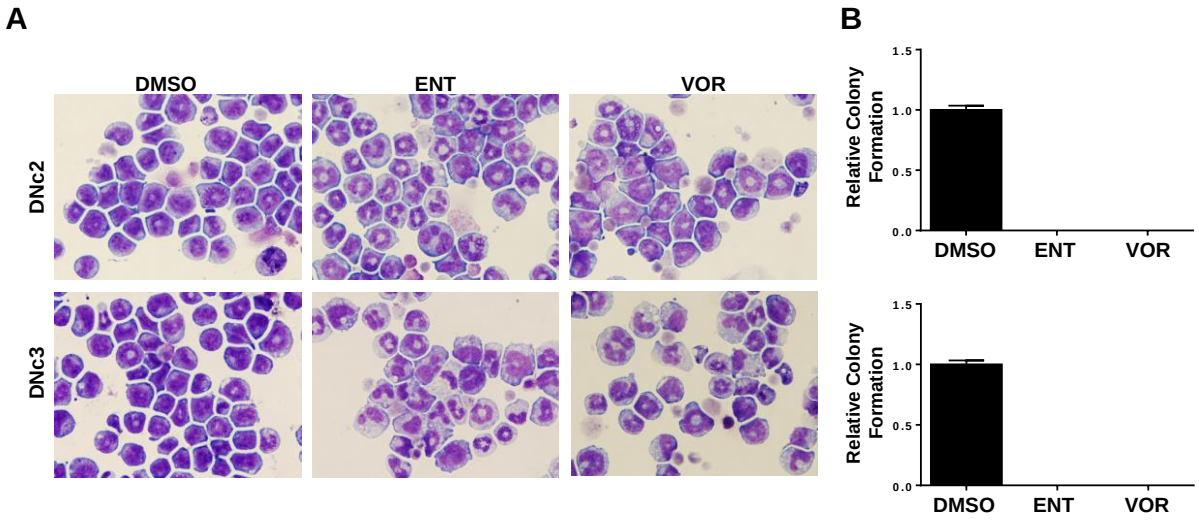

Figure S14. HDAC inhibitors induced differentiation of myeloid progenitors immortalized by SETBP1 activation mutation identified in leukemia patients. **(A)** Representative cytospin of myeloid progenitor cells (DNc2 and DNc3) immortalized by mutant *Setbp1* (harboring activation mutation D868N) after 48hrs of treatment with 1μM of entinostat (ENT), vorinostat (VOR), or control DMSO. **(B)** Mean and SD of colony formation potential of DNc2 (upper panel) and DNc3 (lower panel) cells in the presence of 1μM entinostat, vorinostat or DMSO (n=3 for each treatment).

References

1. Oakley K, Han Y, Vishwakarma BA, Chu S, Bhatia R, Gudmundsson KO*, et al.* Setbp1 promotes the self-renewal of murine myeloid progenitors via activation of Hoxa9 and Hoxa10. *Blood* 2012 Jun 21; **119**(25)**:** 6099-6108.

2. Li M, Riddle SR, Frid MG, El Kasmi KC, McKinsey TA, Sokol RJ*, et al.* Emergence of fibroblasts with a proinflammatory epigenetically altered phenotype in severe hypoxic pulmonary hypertension. *Journal of immunology* 2011 Sep 1; **187**(5)**:** 2711-2722.

3. Larsson P, Ulfhammer E, Magnusson M, Bergh N, Lunke S, El-Osta A*, et al.* Role of histone acetylation in the stimulatory effect of valproic acid on vascular endothelial tissue-type plasminogen activator expression. *PloS one* 2012; **7**(2)**:** e31573.

4. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* 2013 May 30; **368**(22)**:** 2059-2074.